agronomy

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Use of Essential Oils and α-Pinene as Insecticides against *Sitophilus zeamais* and Their Effects on Maize Seed Germination

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Abstract: This study aimed to assess the efficiency of the use of α-pinene and essential oils of *Gaultheria procumbens*, *Juniperus communis*, *Protium heptaphyllum*, and *Protium pallidum* in treating corn seeds (*Zea mays*) under storage conditions for the management of *Sitophilus zeamais*. Contact toxicity, fumigation, repellency, persistence, and residual effects of the targeted essential oil and phytocompound on germination were performed. *G. procumbens* oil, high in methyl salicylate (96%), was the most toxic in contact tests, with an LC_{50} of 26.83 µL/20 g. *P. heptaphyllum* oil, containing 40.1% limonene, was the second most toxic with an LC50 of 45.78 µL/20 g. When test separately, α-pinene was more toxic than *J. communis* oil, which has 67% α-pinene. *P. pallidum* oil, with 31.17% o-cimene, also showed toxicity. In fumigation tests, the toxicity order was *G. procumbens* ≥ *P. heptaphyllum* > α-pinene > *J. communis* > *P. pallidum*. All products were repellent. *G. procumbens* had the longest persistence (71 days), while *J. communis* and α-pinene had shorter persistence. *J. communis* oil and α-pinene did not affect corn seed germination or vigor. The findings are crucial for managing *S. zeamais* in stored maize and determining the appropriate use of natural insecticides without affecting their ability to germinate and grow.

Keywords: botanical insecticides; storage pest; fumigation; contact; repellency; physiological quality

Citation: de Carvalho Brito, R.; de Moura Pádua, L.E.; da Silva, L.R.; Briozo, M.E.O.; Silva, P.R.R.; de Carvalho, L.F.; de Andrade Dutra, K.; Navarro, D.M.d.A.F.; Barbosa, D.R.e.S.; Rojas, M.O.A.I.; et al. Use of Essential Oils and α-Pinene as Insecticides against *Sitophilus zeamais* and Their Effects on Maize Seed Germination. *Agronomy* **2024**, *14*, 2282. https://doi.org/10.3390/agronomy14102282

Academic Editors: Soledad García-Morales and Rebeca G. Betancourt-Galindo

Received: 3 September 2024
Revised: 24 September 2024
Accepted: 25 September 2024
Published: 4 October 2024

1. Introduction

Although more than 37 insect species have been reported as pests associated with stored maize [1,2], the maize weevil [*Sitophilus zeamais* L. (*Coleoptera: Curculionidae*)] holds utmost significance during the processes of drying, transportation, and storage within cultivation in South America [3] and in Eastern and Southern Africa [4].

The pesticides used in stored grain pest control can cause issues for human health and the environment [5]. In addition, numerous insects, which are grain storage pests, have evolved resistance to the fumigant phosphine [6]. Therefore, there is an active need to develop safe techniques and obtain products that have the potential to replace chemical fumigants with natural products.

Coming from the secondary metabolism of plants, essential oils have been used as insecticides and were listed as a promising source due to their worldwide availability and satisfactory cost-effectiveness [7–10]. They are mainly composed of monoterpenes and sesquiterpenes synthesized in the cytoplasm and plastids, representing a complex mixture of organic compounds, some of which represent more than 80% of the composition of oils, which generally characterize their biological activity [11].

The plant species that produce essential oils are distributed throughout the world and in several plant families and has a toxicity potential for insects [12–16]. For example, essential oils from plant species *Gaultheria procumbens* L. (*Ericaceae*) [17,18], *Juniperus communis* L. (*Cupressaceae*) [19], and the genus *Protium* (*Burseraceae*) [20] showed toxicity to pests that attack stored products in previous studies, as well as the compound α-pinene [21–23].

However, the recommendation to use essential oils and their compounds alone for the protection of stored seeds should be made with caution, as several studies have shown that they can cause phytotoxic effects on germination [24,25].

Given these possibilities, it is important to encourage the realization of new research so that more and more plant-based products are studied, both in terms of their toxicity to insect pests and their phytotoxicity to vegetables, thus emphasizing the exploration in a conscious way in all aspects.

Therefore, the objective was to investigate the toxicity of the compound α-pinene and four essential oils [*Gaultheria procumbens* L. (*Ericaceae*), *Juniperus communis* L. (*Cupressaceae*), *Protium pallidum Cuatrec*. (*Burseraceae*), and *Protium heptaphyllum* (*Aubl*.) *March*. (*Burseraceae*)] for *S. zeamais* under storage conditions and their effects on maize seed germination [*Zea mays* L. (*Poaceae*)].

2. Materials and Methods

The Science Center Agrarians—CCA of the Federal University of Piauí—UFPI developed the contact toxicity, fumigation, and repellency experiments, while the Technical College of Teresina—CTT/UFPI's Laboratory of Seeds conducted the germination tests of maize seeds under controlled conditions of temperature and relative humidity. The Federal University of Pernambuco's (UFPE) Chromatography Laboratory, located in the Department of Fundamental Chemistry's Area of Exact and Natural Sciences, was the site of the chromatographic analyses.

2.1. Elimination of Infestation and Seed Moisture Balance

The corn seeds used for the insect rearing stock and experiments underwent a preselection process and were dried. Subsequently, they were stored in bags and plastic, then placed in a freezer at −10 °C for seven days. This step aimed to eliminate any potential insect infestations from the field. After being removed, the seeds were transferred to glass jars and kept in the laboratory for 10 days to achieve hygroscopic equilibrium at 27 °C and 60% relative humidity.

2.2. Sitophilus zeamais Rearing

The rearing stock maintained in the Entomology Laboratory is multiplied for several generations in corn seeds (BR-106) in a glass container or plastic container with a perforated lid to allow gas exchange [26]. The insects were confined for 20 days in containers with corn to carry out the oviposition. Following confinement, the grains were sieved and the insects were discarded. The containers were kept in a breeding room with a temperature controlled at approximately 27 ± 2 °C and a relative humidity of 60 ± 5% until the emergence of adults.

2.3. Essential Oils and α-Pinene

The α-pinene compound with 98% purity was purchased from the company Sigma-Aldrich (São Paulo, Brasil LTDA) and the essential oils of *G. procumbens* and *J. communis* both fruit porvenines were purchased from the company FERQUIMA® Industry and Commerce LTDA (São Paulo, Brasil); the essential oil from the resin of *P. pallidum* was acquired from the company Terra Flor Industria e Comércio de Aromaterapicos LTDA (Alto Paraíso de Goiás, Brasil) and *P. heptaphyllum* was obtained by the process of resin hydrodistillation described below:

The resin was purchased at a local trade in Teresina—PI, with a center of origin in Timon-MA. The oil was extracted from the resin of *P. heptaphyllum* using the hydrodistillation method in a modified Clevenger-type appliance at the Laboratory of Organic Chemistry in the Nature Science Center—CCN [27,28].

Five hundred grams of crushed resin were combined with 3 L of distilled water in a round-bottom flask. The extraction process took place for about 4 h, at a constant temperature (100 °C) to maintain boiling. Over this time, the hydrolate was collected, which was partitioned in a separating funnel for the elimination of the aqueous phase and the recovery of the essential oil. Following the extraction process, the oil was stored in refrigeration at a temperature of −10 °C.

2.4. Gas Chromatography and Mass Spectrometry for the Identification of Compounds

Qualitative analyses were performed using GC/MS from Agilent Technologies (Santa Clara, CA, USA) (5975C Series) equipped with a DB-5 column (60 m × 0.25 mm × 0.25 μm). Quantitative analyses were performed using a GC-FID Thermo Fisher Scientific Ultra Trace (Waltham, MA, USA) equipped with a DB-5 column (30 m × 0.25 mm × 0.25 μm). The analytical conditions were as follows: sample (1 μL of 2000 ppm) injected in split mode (1:20) with injector temperature at 250 °C; oven temperature held initially at 60 °C for 3 min, then increased to 240 °C at 2.5 °C/min and held at 240 °C for 10 min; helium carrier gas flow maintained at 1 mL/min at a constant pressure of 7.0 psi; mass selective detector source and quadrupole temperatures set to 230 °C and 150 °C, respectively; MS obtained at 70 eV and recorded in the range 35–350 m/z at 1.0 scan/s. Each component of the EO was identified by comparison of retention indices obtained by co-injection of the sample with C9–C30 (Sigma-Aldrich—San Luis, MO, USA) linear hydrocarbons and calculated according to the Van den Dool and Kratz [29] equation with those reported in the literature. The volatile compounds were identified by contrast of their experimental mass spectra with that reported in a mass spectra library (MassFinder 4, NIST08, and Wiley RegistryTM 9th) [30] and by confronting their respective experimental retention indexes with those available from the literature (MassFinder 4, NIST08, and Wiley RegistryTM 9th) for an equivalent non-polar column. Quantitative analyses were performed using a GC-FID Thermo Fisher Scientific Ultra Trace (Waltham, MA, USA) equipped with a DB-5 column (30 m × 0.25 mm × 0.25 μm) and the same conditions of analysis in GCMS. (According to [31,32]).

2.5. Contact Toxicity Test

Different concentrations obtained in pre-tests were used for each product: essential oils of *G. procumbens* (4, 8, 10, 13, 20, 26, 35, 48, 55, 65, 90, 100, and 110 μL/20 g), *J. communis* (30, 40, 60, 70, 80, 100, and 110 μL/20 g), *P. pallidum* (30, 50, 60, and 80 μL/20 g), *P. heptaphyllum* (10, 20, 40, 50, 60, 70, 80, 100, and 110 μL/20 g), and the compound majority α-pinene (50, 60, 80, and 130 μL/20 g). Each experimental unit consisted of 20 g of BR-106 corn, treated with the products individually, and a control (no product addition). These products were packaged in 100 mL plastic containers with a perforated lid to allow for gas exchange [26].

The products were impregnated into the corn seeds through a variable-volume automatic micropipette (SCILOGEX). Then, the containers were subjected to manual shaking for one minute to evenly distribute the products in the seeds [33]. After this process, in each repetition, 12 unsexed adults of *S. zeamais* that were 0–15 days old were introduced.

For each treatment, five repetitions were used. The mortality assessment was carried out 48 h after setting up the experiment, with the insects that did not respond to stimuli being considered dead.

2.6. Repellent Effect of Essential Oils and α-Pinene on S. zeamais

The lethal concentrations LC_{15}, LC_{30}, and LC_{50} were used as treatments for repellency based on the results of the contact toxicity tests. Tests were performed in olfactorometer-style arenas made up of two 100 mL plastic containers connected to a central container of the same size by polyethylene tubes in a way that was symmetrical. In one of the containers, 20 g BR-106 corn grains without product was placed (control), and in the other, the same amount of grains was impregnated with the respective concentrations of each treatment using an automatic micropipette and stirred for two minutes. In the central container, 16 unsexed adults (0–15 days old) of *S. zeamais* were placed [33,34]. The experiment was carried out with 10 repetitions, and the number of insects attracted to the grains was measured 24 h after the assembly of the experiment.

2.7. Fumigation Toxicity Tests

Different concentrations were used for each product: oils of *G. procumbens* (100, 200, 300, 500, 600, 700, and 800 µL/L of air), *J. communis* (200, 300, 400, 500, 600, and 700 µL/L of air), *P. pallidum* (350, 400, 500, 600, 700, 900, and 1000 µL/L of air), *P. heptaphyllum* (200, 300, 400, and 500 µL/L), and the chemical compound α-pinene (350, 400, 450, and 700 µL/L). Clear plastic polypropylene containers with lids were utilized to assess the fumigant effect of the products on the adults of *S. zeamais*. For each repetition, 20 unsexed individuals, aged 0–15 days, were placed in these containers. The volume of each container was 100 mL. By affixing strips of filter paper measuring 5 × 2 cm to the underside of the container lids, the products were impregnated using an automatic micropipette. Porous fabric was positioned between the container's lid and contents in an effort to prevent insects from coming into direct contact with the products. The containers were sealed with adhesive tape to prevent the escape of vapors. In the fumigation test, for each treatment, four repetitions were conducted. The assessment of mortality in the test was performed 48 h after the setup of the experiment, with the insects that did not respond to mechanical stimuli considered dead. There was a control treatment (no oil application) [31].

2.8. Persistence of the Insecticidal Effect of Essential Oils and α-Pinene on Stored Seeds

LC_{95} from *G. procumbens*, *J. communis,* and α-pinene (those that exhibited higher toxicity) were used in the contact toxicity tests, aiming to observe for how long the oils and compounds remain with their properties unchanged as insecticides. For the persistence test, 500 g of BR-106 corn seeds were allocated for each product. From this, 400 g were used, divided into four 100 g samples, and each sample was individually treated and homogenized as described earlier in the contact test. The treated samples were then stored in a transparent zip lock bag, wrapped in aluminum foil, and kept under refrigeration at 10 °C until the experiment was set up. The 100 g untreated was used as a control. The experiment was carried out under controlled conditions of a temperature of 27 ± 2 °C and a relative humidity of 60%. Persistence evaluations were made at times 5, 38, 46, 56, and 71 days after seeds were treated. Arrived at the corresponding time, a sample of corn seeds was opened and divided into 5 replicates of 20 g of corn, packed in a 100 mL plastic container with a perforated lid, accompanied by the addition of 12 unsexed adults of *S. zeamais*, 0–15 days of age. After 7 days, the number of insects that were dead was counted.

2.9. Residual Effect of Essential Oils and α-Pinene on the Germination of Stored Seeds

LC_{95} from *G. procumbens*, *J. communis,* and α-pinene were used in the contact toxicity tests for *S. zeamais*, respecting the same evaluation times observed in the persistence test, except the time of 5 days, aiming to observe whether the concentrations found for seed protection influenced their germination. The tests of germination were installed

using four replicates of 50 seeds per treatment. The seeds were sown on Germitest paper previously moistened with distilled water in an amount equivalent to 2.5 times its dry weight. The rolls made and packed in plastic bags were kept in a Mangelsdorf mod. luca-207A at a temperature of 25 °C. The evaluations were performed daily for 7 days, considering the percentage of normal seedlings [35].

The variables were calculated to be as follows:

Total germination (G) is calculated by the formula G = (N/200) × 100, where N = number of germinated seeds at the end of the test. Unit: %.

Germination speed index (GSI) is calculated by the formula GSI = Σ (ni/ti), where ni = number of seeds that germinated in time 'i'; t_i = after test installation; and i = 1 → 7 days. Unit: dimensionless [36].

The formula for calculating average germination time (AGT) is AGT = (niti)/ni, where Ni is the daily number of seeds that germinate, t_i is the incubation period, and days is the unit of measurement.

Average germination speed (AGS) is calculated by the AGS formula = 1/t, where t = average germination time. Unit: days.

Fresh and dry seedling mass: All normal seedlings from the germination test were weighed to obtain the medium fresh weight. To obtain the dry weight, the seedlings were packed in paper bags and taken to the thermoelectric greenhouse of forced air circulation at 80 ± 2 °C for 48 h for drying. The mass was determined on a 0.001 g precision scale, and the results were expressed in grams per seedling evaluated (g/seedling).

2.10. Statistical Analysis and Experimental Design

In all tests, the design used was entirely randomized.

We used PROC PROBIT for the hit test to figure out the concentrations of oils and α-pinene needed to kill 15%, 30%, 50%, and 95% of the population (LC_{15}, LC_{30}, LC_{50}, and LC_{95}). The toxicity ratios (TR) were calculated for each LC individually by dividing the quotient of the product with the highest concentration (LC_{15}, LC_{30}, LC_{50}) and LC_{95} by the toxicity ratios (LC_{50}, LC_{95}) of the remaining products. The evaluations for fumigation were identical to those described previously for contact, with the exception of LC_{50} and LC_{95} and their corresponding toxicity ratios.

To assess repellency, the Chi-square test was employed to interpret the differences in the number of insects attracted to each recipient, as determined by the Proc FREQ ($p < 0.05$).

The experiment methodology for the persistence test consisted of five repetitions and a 4 × 5 factorial design (four products and five storage times). For the product factor, the control condition without products was accounted for. The mortality results (%) were submitted to ANOVA by Tukey's test ($p < 0.05$).

The total germination, GSI, and dry mass data were analyzed in a 4 × 4 factorial scheme (4 products × 4 storage times) with 4 repetitions, where the control, without product, was counted for the factor products. The results were submitted to ANOVA, and means were compared by Tukey's test ($p < 0.05$) individually for each variable.

Dry mass, AGT, and AGS were all subjected to analysis of variance (ANOVA), with evaluations over time and the four treatments (oils, α-pinene, and control) regarded as repeated measures. The means of the treatments were compared using Tukey's test ($p < 0.05$) when the ANOVA results indicated that a particular treatment was significantly different. The data used were considered normal in the statistical analysis.

All analyzes were performed using the Statistical Program SAS version 8.02 [37].

3. Results

3.1. Compound Identification

The composition of the oils varied among the different species, and the major compounds were identified. Methyl salicylate (calculated retention index: 1202; literature retention index: 1190) is an ester and was the only compound identified for *G. procumbens* with 96%. In the essential oil of *J. communis,* a total of 33 compounds were found, mostly

monoterpenes (91%) and sesquiterpenes (6.72%). The compounds α-pinene, β-pinene, and limonene were the most abundant, with 67, 13, and 4%, respectively. For the oil of *P. heptaphyllum*, six compounds were quantified, distributed between monoterpenes (82.72%) and oxygenated monoterpenes (2.93%), where the most abundant were limonene (66.30%), δ-3-carene (11.22%), and α-terpineol (2.93%). The oil of *P. pallidum* has in its composition 88.76% of monoterpenes, 5.49% of oxygenated monoterpenes, and 1.51% of sesquiterpenes, distributed together in 27 compounds, where the most abundant were o-cymene (31.17%), β-phelandrene (25.9%), and α-pinene (16.99%) (Table 1) (Figure S1A–D).

Table 1. Chemical composition of essential oils from *Juniperus communis*, *Protium heptaphyllum*, and *Protium pallidum*.

	Compound [a]	*Juniperus communis*			*Protium heptaphyllum*		*Protium pallidum*	
		RI [b]	RI [c]	(%)	RI [c]	(%)	RI [c]	(%)
1	Tricyclene	921	918	0.06	-	-	-	-
2	α-Thujene	924	925	0.34	-	-	926	0.27
3	α-Pinene	932	930	67.03	931	1.45	932	16.99
4	Camphene	946	945	0.63	-	-	946	0.57
5	Sabinene	972	-	-	969	1.67	-	-
6	β-Pinene	974	973	12.85	-	-	975	2.83
7	Menthene<3-p->	984	-	-	-	-	983	0.29
8	Myrcene	988	990	3.95	-	-	992	0.11
9	α-Phellandrene	1002	1002	0.18	1003	2.08	1004	5.63
10	δ-3-Carene	1008	-	-	1009	11.22	1010	0.43
11	α-Terpinene	1014	1015	0.13	-	-	1016	2.45
12	o-Cymene	1022	1023	0.81	-	-	1025	31.17
13	Limonene	1024	1027	4.22	1027	66.30	-	-
14	β-Phellandrene	1025	-	-	-	-	1029	25.90
15	y-Terpinene	1054	1058	0.51	-	-	1059	0.21
16	Terpinolene	1086	1087	0.58	-	-	1088	0.91
17	α-Pinene oxide	1099	1096	0.08	-	-	-	-
18	Menth-2-en-1-ol<cis-p>	1118	-	-	-	-	1122	0.08
19	E-Pinocarveol	1135	1137	0.08	-	-	-	-
20	Terpineol<cis-dihydro-α->	1143	-	-	-	-	1144	1.59
21	Terpinen-4-ol	1174	1176	1.01	-	-	1178	0.29
22	Cryptone	1183	-	-	-	-	1186	0.66
23	α-Terpineol	1186	1190	0.43	1191	2.93	1191	2.25
24	Myrtenol	1194	1196	0.11	-	-	-	-
25	Piperitone	1249	-	-	-	-	1255	0.46
26	Linalool acetate <dihydro->	1272	-	-	-	-	1276	0.16
27	Isobornyl acetate	1283	1386	0.14	-	-	-	-
28	α-Cubebene	1348	1350	0.28	-	-	1351	0.20
29	α-Copaene	1374	1376	0.19	-	-	1378	0.69
30	β-Elemene	1389	1392	0.20	-	-	-	-
31	β-Longipinene	1400	1405	1.25	-	-	-	-
32	E-Caryophyllene	1417	1420	2.72	-	-	-	-
33	α-Humulene	1452	1452	0.49	-	-	1456	0.09
34	y-Muurolene	1478	1477	0.16	-	-	-	-
35	Germacrene D	1480	1482	0.32	-	-	-	-
36	γ-Himachalene	1481	-	-	-	-	1486	0.21
37	β-Selinene	1489	1487	0.06	-	-	-	-
38	Valencene	1496	1496	0.14	-	-	-	-
39	α-Muurolene	1500	1501	0.13	-	-	1503	0.07
40	y-Cadinene	1513	1515	0.16	-	-	1517	0.09
41	δ-Cadinene	1522	1524	0.61	-	-	1526	0.16
42	Germacrene B	1559	1558	0.17	-	-	-	-
43	Caryophyllen oxide	1582	1584	0.32	-	-	-	-

Table 1. *Cont.*

Compound [a]	*Juniperus communis*			*Protium heptaphyllum*		*Protium pallidum*	
	RI [b]	RI [c]	(%)	RI [c]	(%)	RI [c]	(%)
Monoterpene			91.32		82.72		88.76
Oxygenated Monoterpene			1.85		2.93		5.49
Sesquiterpene			6.72		-		1.51
Oxygenated Sesquiterpene			0.32		-		-
Total			100		86.65		95.76

[a] Constituents listed in order of elution on DB-5 nonpolar column, GC-FID detector; [b] Linear Retention Indices from the literature (Adams, 2005) [30]. [c] Linear Retention Indices calculated through the retention times in relation to the n-alkanes series (C8–C25); % Percentage of compound in the essential oil.

3.2. Contact Toxicity Test

The toxicity of oils and α-pinene was determined using concentration–mortality curves, where there was a variation in lethal concentrations between the products tested with LC_{15}, LC_{30}, LC_{50}, and LC_{95} of 10.62, 16.79, 26.83, and 116.79 (µL/20 g of seeds) for *G. procumbens*; 54.97, 66.15, 79.9, and 144.99 for *J. communis*; 31.01, 37.59, 45.78, and 84.96 for *P. heptaphyllum*; 29.18, 40.96, 57.98, and 172.35 for *P. pallidum*; and 41.60, 46.16, 51.35, and 71.74 for α-pinene, respectively (Table 2).

Table 2. Contact toxicity of essential oils and α-pinene on adults of *Sitophilus zeamais*.

Treatment	N	DF	Slope ± SE	LC_{15} (CI) *	LC_{30} (CI) *	LC_{50} (CI) *	TR_{50}	LC_{95} (CI) *	TR_{95}	χ^2	*p* [1]
Gaultheria procumbens	780	11	2.57 ± 0.15	10.62 (8.90–12.29)	16.79 (14.74–18.80)	26.83 (24.26–29.55)	2.98	116.79 (98.95–142.99)	1.47	5.09	0.92
Protium heptaphyllum	240	2	6.12 ± 0.69	31.01 (26.39–34.62)	37.59 (33.48–40.92)	45.78 (42.21–49.14)	1.74	84.96 (75.76–100.61)	2.02	0.03	0.98
Protium pallidum	540	7	3.47 ± 0.31	29.18 (24.30–33.40)	40.96 (36.19–45.17)	57.98 (53.25–62.91)	1.37	172.35 (144.91–219.30)	-	7.51	0.37
Juniperus communis	420	5	6.36 ± 0.87	54.97 (43.94–62.37)	66.15 (57.09–73.29)	79.97 (72.08–89.52)	-	144.99 (120.45–208.53)	1.18	10.33	0.06
α-pinene	240	2	11.33 ± 1.89	41.60 (35.57–45.26)	46.16 (41.16–49.18)	51.35 (47.91–53.89)	1.55	71.74 (66.66–81.94)	2.40	3.81	0.14

* µL/20 g, N = number of insects, DF = degree freedom, SE = standard error, CI = confidence interval, TR = toxicity ratio, χ^2 = Chi-square., 1 = Probability ($p > 0.05$).

The Probit model was suitable for analyzing concentration–mortality data, with low χ^2 values and high *p* values for each concentration–mortality curve ($\chi^2 < 10.33$ and $p > 0.05$). There was a variation in the slope of the products, from 2.57 to 11.33, indicating some toxicological heterogeneity among the products tested, where higher values of the slope of the curve indicate that small variations in the concentration of oils provide large variations in mortality (Table 2).

The lowest lethal concentrations were found in α-pinene (LC_{30}: 46.16; LC_{50}: 51.35; and LC_{95}: 71.74 µL/20 g) when compared to those found in *J. communis* oil (LC_{30}: 66.15; LC_{50}: 79.97; and LC_{95}: 144.99 µL/20 g), showing that this monoterpene is possibly the cause of mortality caused by *J. communis* oil on *S. zeamais* (Table 2).

3.3. Repellent Effect of Essential Oils and α-Pinene on S. zeamais

The number of adults of *S. zeamais* attracted to corn seeds treated with the essential oils of *G. procumbens*, *J. communis*, *P. heptaphyllum*, *P. pallidum*, and α-pinene was significantly lower ($p < 0.05$) when compared with the untreated seeds, indicating that they were repellent. All products exhibited repellent activity, regardless of the concentration tested (Figure 1).

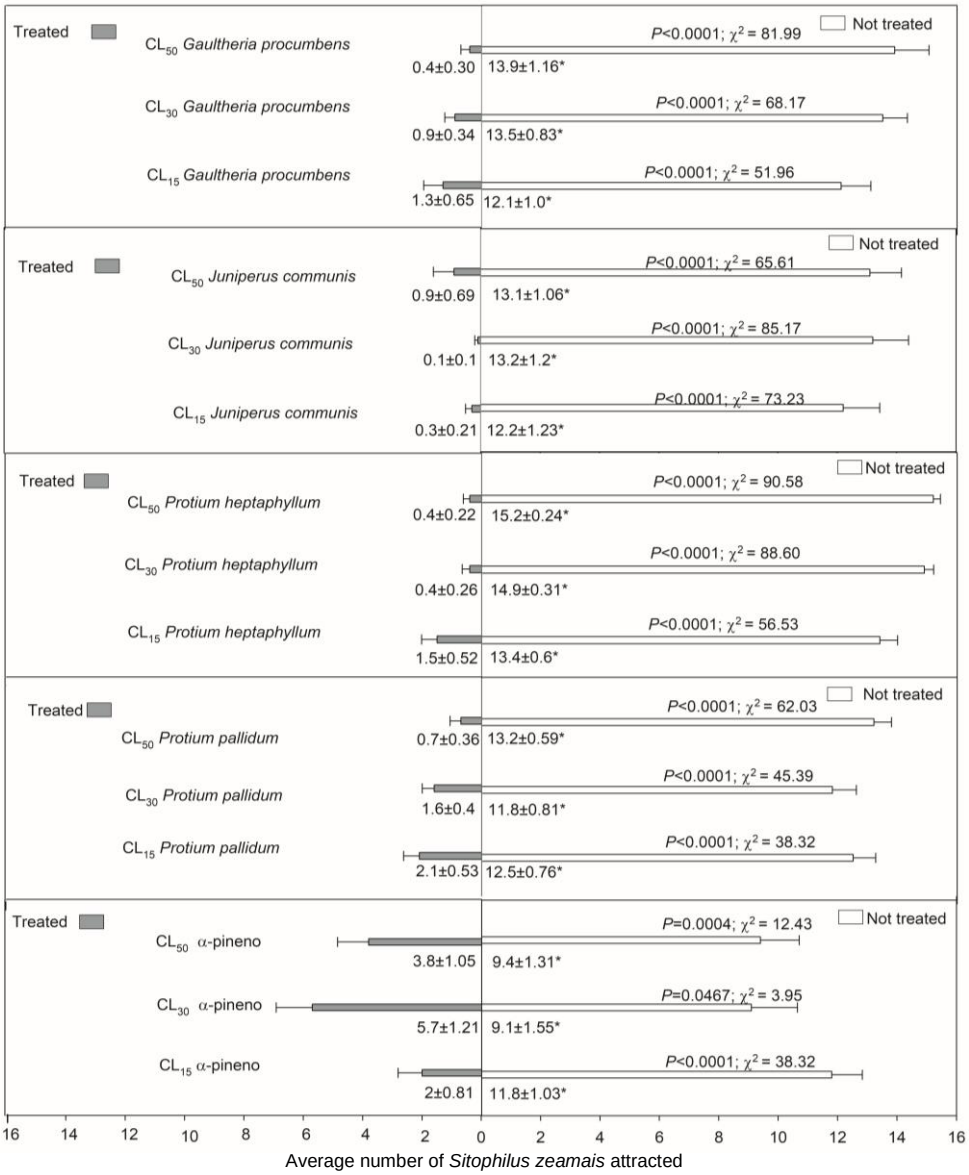

Figure 1. Number of *Sitophilus zeamais attracted* (n = 480) in corn kernels treated and not treated with the oils of *Gaultheria procumbens*, *Juniperus communis*, *Protium heptaphyllum*, *Protium pallidum*, and α-pinene. * Significant by the Chi-square test ($p < 0.05$).

3.4. Fumigation Toxicity Tests

The oils showed the following order of toxicity by fumigation: *G. procumbens* ≥ *P. heptaphyllum* > α-pinene > *J. communis* > *P. pallidum* for LC_{50} (Table 3). The essential oil of *G. procumbens* had a higher toxicity ratio (TR_{50}) when compared to the oil with lower toxicity, *P. pallidum*. Although the essential oil of *G. procumbens* has a lower LC_{50} (231.65), we cannot indicate it as more toxic due to the overlapping of the confidence interval with the oil of *P. heptaphyllum* (Table 3).

The α-pinene compound showed a higher slope (10.25 ± 1.38), showing that small increments in concentration ensure high mortality responses, but the other products showed a slight variation in the slope from 4.05 to 6.24, indicating a toxicological difference between the products tested (Table 3). The compound α-pinene (67.03%) is the main compound in *J. communis* (Table 1). When tested individually, it caused a greater fumigant effect on *S. zeamais;* therefore, it has a lower lethal concentration in relation to the mixture of compounds in the essential oil of *J. communis;* therefore, minor compounds in the oil may

interact antagonistically with α-pinene, leading to an increase in the lethal concentration of the essential oil. (Table 3).

Table 3. Fumigation toxicity of essential oils and α-pinene on adults of *Sitophilus zeamais*.

Treatment	N	DF	Slope ± SE	LC_{50} (CI) *	TR_{50}	LC_{95} (CI) *	TR_{95}	χ^2	p [1]
Gaultheria procumbens	560	5	4.05 ± 0.28	231.65 (219.53–252.45)	2.63	589.05 (525.72–677.40)	1.95	7.95	0.15
Protium heptaphyllum	320	2	6.24 ± 0.98	280.29 (188.67–353.23)	2.18	514.27 (393.07–1625)	2.23	5.13	0.07
Protium pallidum	560	5	5.98 ± 0.43	611.09 (583.22–640.70)	-	1150 (1049–1297)	-	9.00	0.10
Juniperus communis	480	4	4.91 ± 0.44	493.38 (464.54–526.03)	1.23	1067 (929.30–1294)	1.07	3.59	0.46
α-pinene	320	2	10.25±1.38	407.19 (392.18–422.97)	1.50	589.74 (541.79–679.26)	1.95	1.70	0.42

* µL/20 g, N = number of insects, DF = degree freedom; SE = standard error, CI = confidence interval, TR = toxicity ratio, χ^2 = Chi-square., 1 = Probability ($p > 0.05$).

3.5. Persistence of the Insecticidal Effect of Essential Oils and α-Pinene on Stored Seeds

There was a significant interaction between storage time and treatments ($p < 0.05$) regarding toxicity. The oil from *G. procumbens* was the most persistent, causing 100% mortality regardless of the storage period, thus pointing out that the insecticidal effect can last up to 71 days if storage occurs under the same conditions foreseen in the proposed methodology (Table 4). However, *J. communis* oil caused 100% mortality only until the fifth day of storage. From the 38th day on, the insecticide effect decreased, causing about 30% mortality in the target insect at 71 days, showing that the residual effect decreases over time. The compound, α-pinene, caused the least mortality, even in the first evaluation period (5 days), so it has low persistence (Table 4).

Table 4. Toxicity (percentage) of essential oils and α-pinene on *Sitophilus zeamais* after different storage periods of treated seeds.

Treatment	µL/20 g [1]	Storage Period (Days)				
		5	38	46	56	71
Control	0.0	0.0 cA	0.0 dA	0.0 dA	0.0 cA	0.0 dA
Gaultheria procumbens	116.79	100.0 aA	98.3 aA	100.0 aA	100.0 aA	100.0 aA
Juniperus communis	144.99	100.0 aA	65.0 bB	63.3 bB	46.6 bC	30.0 bD
α-pinene	71.74	16.6 bB	15.0 cB	41.6 cA	38.3 bA	16.6 cB

[1] Concentrations; Means followed by the same letter do no differ significantly in the column (lowercase) and in the line (uppercase) by Tukey's test ($p < 0.05$).

3.6. Residual Effect of Essential Oils and α-Pinene on the Germination of Stored Seeds

There was a significant interaction between storage periods and treatments on the impact of germination and fresh mass production ($p < 0.05$). The oil of *G. procumbens* decreased the germination percentage ($p < 0.05$), delayed the germination speed of corn seeds ($p < 0.05$), and decreased the fresh mass weight ($p < 0.05$) when compared with the control treatment, indicating a loss of viability and vigor. Despite the statistical differences caused by the essential oil of *J. communis* and the compound α-pinene in comparison with the control, the values are still considered satisfactory, considering the Brazilian legislation (Table 5).

The average germination time and the average germination speed of corn seeds were not affected when the seeds were subjected to different periods of exposure to *G. procumbens* and *J. communis* oils and to the compound α-pinene ($p > 0.05$) (Table 6). On the other hand, the dry matter of maize seedlings was affected when the seeds were submitted to treatments

for 46, 56, and 71 days ($p > 0.05$). However, *G. procumbens* at times 46, 56, and 71 and *J. communis* at time 71 did not differ from the control ($p > 0.05$) (Table 6).

Table 5. Germination (G), germination speed index (GSI), and fresh mass weight (FM) of maize seedlings subjected to different treatments after four seed storage periods.

Variable	Treatment	Storage Pediod (Days)			
		38	46	56	71
G (%)	Control	99 aA	99 aA	99 aA	99 aA
	Gaultheria procumbens	44 bC	90 bA	80 bB	75 bB
	Juniperus communis	92 aAB	85 bB	91 aAB	97 aA
	α-pinene	99 aA	99 aA	96 aA	99 aA
GSI	Control	16.47 aA	16.47 abA	16.47 aA	16.47 aA
	Gaultheria procumbens	7.25 bC	14.97 bcA	13.28 bB	12.47 bB
	Juniperus communis	15.3 aAB	14.0 cB	15.1 aAB	16.1 aA
	α-pinene	16.58 aA	16.56 aA	16.0 aA	16.52 aA
FM (g/seedling)	Control	1.04 aA	1.04 bA	1.04 aA	1.04 aA
	Gaultheria procumbens	0.87 bB	1.05 abA	0.89 bB	0.90 bB
	Juniperus communis	1.09 aAB	1.10 abA	1.01 aB	1.03 aAB
	α-pinene	1.10 aA	1.13 aA	1.01 aB	1.09 aAB

Means followed by the same letter do not differ significantly in the column (lowercase) and in the line (uppercase) by Tukey's test ($p < 0.05$) for each variable analyzed separately.

Table 6. Average germination time (AGT), average germination speed (AGS), and dry mass (DM) of maize seedlings subjected to different treatments and storage time.

Variable	Treatment	Storage Period (Days)			
		38	46	56	71
AGT (days)					
	Control	3.00 (NS)	3.005 (NS)	3.005 (NS)	3.005 (NS)
	Gaultheria procumbens	3.06	3.005	3.042	3.034
	Juniperus communis	3.00	3.042	3.005	3.010
	α-pinene	3.00	3.005	3.000	3.015
	CV	1.44	0.88	0.90	0.79
AGS					
	Control	0.332 (NS)	0.332 (NS)	0.332 (NS)	0.332 (NS)
	Gaultheria procumbens	0.326	0.332	0.328	0.329
	Juniperus communis	0.332	0.328	0.332	0.332
	α-pinene	0.333	0.332	0.333	0.331
	CV	1.38	0.87	0.88	0.78
DM (g/seedling)	Control	0.270 (NS)	0.270 a	0.270 a	0.270 ab
	Gaultheria procumbens	0.273	0.267 ab	0.254 ab	0.285 a
	Juniperus communis	0.262	0.253 b	0.251 b	0.274 ab
	α-pinene	0.253	0.253 b	0.249 b	0.268 b
	CV	3.98	2.93	2.95	2.75

NS: not significant. Means followed by unequal letters in the column differ statistically from each other by Tukey's test ($p < 0.05$).

4. Discussion

The variation in the composition of essential oils reflects the sum of one or more interfering factors. Methyl salicylate was the only compound identified in *G. procumbens*, accounting for 96% of the oil composition. Methyl salicylate has been reported as the major compound of *G. procumbens* essential oil with a percentage of 96.90 [38] and 96.61% [17], thus agreeing with the results found in this research.

The monoterpene α-pinene (67%) was the major compound observed in *J. communis* oil. The essential oil from *J. communis* berries harvested in the Abruzzo, Lazio and

Molise National Park (PNALM, Central Italy) has 90 different compounds, where the most abundant monoterpene hydrocarbon was α-pinene (13.43–32.34%) [39]. The essential oil of *J. communis* var. saxatilis from plant material collected in the Stara Planina mountain, Serbia has α-pinene (23.61%) as the majority, followed by the other compounds; δ-cadinene (10.71%), sabinene (9.53%), α-muurolene (6.58%), and γ-cadinene (5.87%) were the other most dominant compounds [40]. The variation in the composition of the essential oil of the same species from different regions can be affected by changes in environmental conditions, such as temperature, light, nutrient status, or water availability in summer or winter, which can cause a metabolic imbalance that can affect biosynthetic pathways [41]. Possibly, the variation in the quantification of α-pinene in *J. communis* oil is due to the location of the collection origin of the plant material for essential oil extraction.

Limonene (66.30%) and o-cymene (31.17%) were the major compounds identified in the oils of *P. heptaphyllum* and *P. pallidum*, respectively. Essential oils from species of the same genus can have different chemical compositions [42]. Studies that characterize the composition of essential oils of the *Protium* genus are still scarce; however, the species of the *Protium* genus produce secondary metabolites with different types of terpenes, with more than 100 different mono and sesquiterpenes characterized [43–45]. Generally, some monoterpenes, such as limonene, α-pinene, α-phelandrene, sabine, terpinolene, and p-cimene, are part of the essential oil composition of *P. heptaphyllum* [46]; however, the major compounds vary, being terpinolene, p-cimene [45–47], or limonene [48], whereas for *P. pallidum*, the γ-elemene is the majority compound [49]. The presence of a certain compound in the essential oil can be directly linked to several factors, such as the high and low boiling points, where many compounds are simply 'lost' due to the nature of the process and restrictions on distillation time [50]. In addition, endogenous factors (anatomical and physiological characteristics of plants and the biosynthetic pathways of volatiles) and exogenous factors (light, precipitation, cultivation site, soil, and seasonal variation) can be responsible for such variation of compounds in essential oils [51]. Therefore, the composition of an essential oil can vary greatly for species of the same genus and for identical species from collections in locations with relevant edaphoclimatic characteristics.

Isolated compounds and essential oils from plants of botanical families similar to those studied here have been reported to be toxic to several insect pests. For instance, essential oil from *Gaultheria procumbens* demonstrated toxicity against stored grain pests, such as *Sitophilus oryzae* L. and *Rhyzopertha dominica* Fabr., by inhibiting acetylcholinesterase (AChE) activity. Kiran and Prakash (2015) [17] found that the in vivo inhibition of AChE activity in these pests ranged between 6.12% and 27.50% when exposed to lethal and sublethal doses of this essential oil. The same effect was seen when adults of *S. oryzae* were exposed to oil from *Gaultheria fragrantissima* Wall. (*Ericaceae*) [52]. The essential oil from *J. communis hemisphaerica* was toxic against *R. dominica* and *Tribolium castaneum* Herbst., being a potential control agent [53]. Previous research demonstrated that the essential oil of *P. heptaphyllum*, rich in limonene, was toxic upon contact with *Callosobruchus maculatus* Fabr. [20]. *S. zeamais* and *T. castaneum* [21] and *Lasioderma serricorne* F. [54] reported mortality caused by the hydrocarbon monoterpene present in many essential oils, α-pinene. Therefore, the products tested here are promising sources of contact insecticides.

There was a variation in the slope values of the toxicity curves for the essential oils, ranging from 2.57 to 11.33, indicating a degree of toxicological heterogeneity among the oils tested. Adult insect mortality can be attributed to the toxicity of the essential oil contact or the abrasive effect on the pest cuticle [55], which can also interfere with the respiratory mechanism of the insect [56–58]. Another aspect clarifies that the contact toxicity of essential oils or isolated compounds can vary based on the susceptibility of different insect species that attack stored grains [59], where the target population comes from, and whether it has high or low resistance to the products [60].

The oil from *G. procumbens* was the most toxic to *S. zeamais*, presenting the highest TR_{50} of 2.98 when compared to the oil with lower toxicity, *J. communis* (Table 3). In the present study, the essential oil of *G. procumbens* contains about 96% methyl salicylate. This

compound, which is naturally a volatile compound induced by herbivores in a plant, has functions such as attracting, repelling, or preventing target organisms, depending on the concentration [61]. Therefore, this compound has a high chance of causing the toxicity of *G. procumbens* in *S. zeamais*. The essential oil of *G. procumbens*, containing mainly methyl salicylate (96.61%) at concentrations close to those studied here, caused alterations in the activity of superoxide dismutase (SOD) and catalase (CAT) in *S. oryzae* and *R. dominica*, where this response was concentration-dependent [17]. Probably, *G. procumbens* oil has the same mechanism of action on *S. zeamais*, mainly affecting the activity of enzymes that act in detoxification processes such as SOD and CAT in insects.

When tested alone, the majority compound of *J. communis*, α-pinene, causes greater toxicity to *S. zeamais*, as it has a lower lethal concentration compared to the mixture of compounds present in the essential oil (Table 2). The topical toxicity of α-pinene has been proven in *S. zeamais* and *T. castaneum* [21]. α-pinene and the essential oil of *Haplophyllum dauricum* (L.) G.Don (*Rutaceae*) with 12.24% of α-pinene and 42.37% of β-pinene present insecticidal contact activity on *T. castaneum* and *L. serricorne*, respectively. The oil in question has promising potential as an ecological botanical insecticide [54]. The essential oil of *Cupressus lusitanica* Mill. (*Cupressaceae*), with 24% α-pinene in its composition, showed toxicity via contact against three stored grain pests (*T. castaneum*, *Acanthoscelides obtectus* Say and *S. zeamais*) [62]. In general, the insecticidal effect of essential oils results from the inhibitory action of acetylcholinesterase (AChE) caused especially by monoterpenes [63,64]. The α-pinene compound is a hydrocarbon monoterpene that already had its structure and activity related to the inhibition of acetylcholinesterase (AChE), which is stronger when compared to alcohols and ketones [65,66]. Generally, monoterpenes have strong contact and fumigation toxicity to insects due to their lipophilic and volatility properties [67], which is a possible justification for α-pinene being more toxic to *S. zeamais* than the essential oil of *J. communis*.

Essential oils from *G. procumbens*, *J. communis*, *P. heptaphyllum*, *P. pallidum*, and α-pinene exhibited repellent activity on *S. zeamais* regardless of the concentration tested. Sublethal effects of essential oils, such as repellency, have been investigated for various stored grain pests. Sublethal concentrations may have varying toxicity depending on the stage of development of the insect; the methodology of application used, whether contact, fumigation, or repellency; and the extraction method of the active ingredient [68]. Essential oils of *Zingiber officinale* Roscoe (*Zingiberaceae*) and *Piper cubeba* L. (*Piperaceae*) and two pure natural terpenes, α-pinene and β-caryophyllene, have a repellent effect on adults of *S. oryzae* in sublethal concentrations [22].

Essential oils containing α-pinene in considerable amounts are seen as promising sources of pest repellents for stored products. The repellent activity of the essential oil of *Pistacia lentiscus* L. (*Anacardiaceae*), whose α-pinene is the major compound, has been proven for several stored grain pests, when the calculated RD_{50} (Repellent concentration that repels 50% of exposed insects) was 0.010, 0.037, and 0.025 μL cm^{-2} for *S. zeamais*, *R. dominica*, and *Tribolium confusum* J. du Val, respectively, whereas α-pinene required 0.262, 0.706, and 0.225 μm cm^{-2} [69]. The investigation of low lethality concentrations, both of essential oils and of isolated compounds that present a repellent effect, demonstrates a good possibility of using effective concentrations against *S. zeamais*. Since α-pinene is the major compound of the essential oil of *J. communis*, we can conclude that this isolate is possibly responsible for the repellent activity observed in *J. communis*. As seen in oils, α-pinene is repellent, and this effect can be considered sublethal.

The most explored control method in search of botanical insecticides is fumigation, as the most commonly used commercial product, phosphine, is applied in this way. Several researchers have listed essential oils and their compounds as promising fumigants for species of the *Sitophilus* genus. Both *G. procumbens* oil (LC_{50} of 58.62 and LC_{90} of 89.79 μL/L) and its major methyl salicate (LC_{50} of 63.49 and LC_{90} of 110.82 μL/L) have insecticidal activities by fumigation on *S. oryzae*. Both are suitable alternatives for the formulation of insecticides against stored grain pests [17]. However, *G. procumbens* essential oil from

Ecosafe Natural Products Inc. (Saanichton, BC, Canada) showed a low LC_{50} over *S. oryzae* by fumigation (6.78 µL/L of air) [18]. In our results, a higher LC_{50} was needed (231.65 µL/L) when compared to the previously cited works. The toxicological variation of *G. procumbens* oil used in pest management of the *Sitophilus genus* seems to be related to different levels of susceptibility of the insects in question. The inhibition of acetylcholinesterase (AchE) enzyme activity was observed in adults of *S. oryzae* when fumigated with sublethal concentrations of *Z. officinale* and *P. cubeba* essential oils, α-pinene, and β-caryophyllene, alone or in sublethal binary combinations [22]. These essential oils and pure compounds, and those tested here in this work, probably induce toxicity by inhibiting the activity of the acetylcholinesterase enzyme.

P. heptaphyllum essential oil is rich in limonene and contains other compounds such as α-terpineol, α-pinene, and p-cymene. These compounds were also identified in the essential oil of *Melaleuca alternifolia* Chell. (*Myrtaceae*), whose fumigant activity against *S. zeamais* was proportional to the increase in the concentration used and significantly inhibited important enzymes such as acetylcholinesterase (AChE), glutathione S-transferase (GST), and carboxylesterase (CarE) [70]. Compounds such as α-terpineol, α-pinene, limonene, and p-cimene may be possible mortality factors for *P. heptaphyllum* essential oil, as they are capable of interfering with important groups of enzymes that are directly linked to the insecticidal mechanism of the products here tested.

The oil of *G. procumbens* was the most persistent and caused 100% mortality in *S. zeamais* up to 71 days after application. The persistence of a particular essential oil or isolated compound is directly linked to the inherent physicochemical properties of each one. Essential oils generally have low persistence, but several studies have identified controversies for this characteristic. Essential oils of *Clausena anisata* (Willd.) Hook. f. ex Benth (*Rutaceae*) and *Plectranthus glandulosus* Hook. f. (*Lamiaceae*) caused 100% mortality in *S. zeamais* until the fourth day after exposure via fumigation, but this stability was lost and significantly decreased until the twentieth day [71]. The essential oil of *Croton pulegiodorus* Baill (*Euphorbiaceae*) had a residual effect on lethal concentrations (LC_{50} and LC_{90}) found for each population of *S. zeamais*, as it affected the emergence of this insect even after 60 days of corn grain storage treated [60]. Essential oils from *Piper hispidinervum* C. DC. (*Piperaceae*), *Melaleuca leucadendron* L. (*Myrtaceae*), *Eugenia uniflora* L. (*Myrtaceae*), *Schinus terebinthifolius* Raddi. (*Anacardiaceae*), *Piper marginatum* Jacq. (*Piperaceae*), and the compound eugenol caused high mortality in *S. zeamais* immediately after its application, but the mortalities decreased with the increase in the exposure period [72]. Being basically composed of an ester (methyl salicylate), the oil of *G. procumbens* persisted longer because its composition was different from that normally found in essential oils.

Gaultheria procumbens had greater persistence due to its composition, probably consisting mostly of less volatile compounds; however, several essential oils have high volatility, which is one of the main characteristics of these compounds. The *J. communis* oil caused 100% mortality only until the fifth day of storage, and the compound, α-pinene, was the least persistent, causing low mortality in the early stages. The insecticidal activity of *J. communis* decreased rapidly because its compounds are plant molecules belonging to groups of monoterpenes and sesquiterpenes, which are volatile due to their photolability [73]. In addition, the rapid deterioration of monoterpene hydrocarbons such as sabinene, 1.8 cineole, and α-pinene, as well as alcoholic compounds, is due to the high rate of oxidation of these essential oils [56,74], justifying the low persistence of these products in our results.

The oil of *G. procumbens* decreased the germination percentage, delayed the germination speed of corn seeds, and decreased the fresh mass weight when compared to the control treatment. However, methyl salicylate, which is the major compound of *G. procumbens* oil, has been used to improve rice seed germination and seedling growth [75]. Another important fact is that methyl salicylate can be converted to salicylic acid by the enzymatic action of carboxyl methyltransferase, and that the role of salicylic acid in seed germination has been controversial, as there are conflicting reports suggesting that it can inhibit germination or increase seed germination. Seed vigor [76] and corn germination, for ex-

ample, were completely inhibited by salicylic acid concentrations ranging from 3 mM to 5 mM [77], and this possible effect is directly linked to oxidative stress by the increase in hydrogen peroxide levels caused by salicylic acid and by increasing the enzymatic activity of Zn-superoxide dismutase and hydrogen peroxide inactivation by degrading enzymes, catalase, and ascorbate peroxidase [78]. Interestingly, when low concentrations are applied exogenously, salicylic acid significantly improves Arabidopsis seed germination and seedling establishment under different conditions of abiotic stress [79,80]. Possibly, the high concentration of methyl salicylate present in *G. procumbens* oil and the possibility of its conversion into salicylic acid may be responsible for the loss of quality in corn seeds.

Seeds treated with the essential oil of *J. communis* and the compound α-pinene remained with a germination percentage above 85% and the germination speed index, as well as the fresh mass weight, were not affected when compared to the control. According to the standard established by Brazilian national agencies, the minimum acceptable germination percentage for maize seed lots is 85% [24], which shows that *J. communis* oil and the compound α-pinene used for the management of *S. zeamais* can be used to protect grains and seeds without affecting their quality.

The use of essential oils in seed treatment, whether to protect fungi or insect pests, can cause deleterious effects on germination due to the phytotoxic effects they cause, but there are exceptions. The essential oils of *Eucalyptus citriodora* Hook (*Myrtaceae*) and *Eucalyptus camaldulensis* Dehnh, when tested for antifungal activities, individually or in binary mixtures, did not affect the germination (>85%) of maize seeds from the Avaré lot, but the essential oil of *E. citriodora* was harmful to the Bernardinho lot because it presented a germination percentage of 72%, preventing its use for seed treatment, according to the standardized standard (85%) by the Ministry of Agriculture, Livestock, and Supply (MAPA) [24]. Essential oils of clove (*Syzygium aromaticum* L.) (*Myrtaceae*) and vatica (*Vatica diospyroides* Symington) (*Dipterocarpaceae*) strongly inhibited maize seed germination, depending on the concentration used; however, *Vatica* oil is more phytotoxic than clove oil [81]. Except for *G. procumbens* oil, data on the average germination percentage tell us that neither the other products nor the storage times are capable of interfering with the viability of corn seeds, which can be used in the management of *S. zeamais* without any undesirable effects.

The dry mass of the grains was only affected after 46 days of storage; however, the variation in phytotoxicity of *J. communis* for dry mass was not linear. Essential oils are mostly made up of monoterpenes, which are a group of compounds with many different functional groups and optical isomers of certain compounds. These isomers can exhibit differential properties, inhibiting or not inhibiting germination [82]. Possibly, the observed variation is directly linked to factors such as oil composition, concentration, and exposure time.

Some oils may not affect the germination percentage but may affect other variables such as average germination time, germination speed, and seedling mass weight, among other variables that may signal the loss of vigor in seed lots. Corn seeds treated with essential oils of the *Croton heliotropiifolius Kunth* species. (*Euphorbiaceae*), *C. pulegiodorus,* and *Ocimum basilicum* L. (*Lamiaceae*) did not have their percentage of germination affected, but ESI (Emergency Speed Index) and ESC (Emergency Speed Coefficient) were obtained after treatment with oil. *O. basilicum* differed from the other treatments [83]. These data corroborate those observed in this work.

The essential oil of *J. communis* and its major α-pinene did not affect the germination percentage, GSI, fresh mass weight, AGT, and AGS. When α-pinene stereoisomers were added to corn seeds, the results were the same. There was no difference in seed vigor based on germination speed compared to the control [82]. The non-interference in variables such as germination percentage, GSI, fresh mass weight, AGT, and AGS is due to the low persistence of *J. communis* oil and α-pinene monoterpene, and its greater volatility when compared to *G. procumbens* oil.

All oils had a lethal and sublethal effect on *S. zeamais* via contact, and the compound α-pinene present in the essential oil of *J. communis* is possibly the cause of mortality in this oil, as it presented lower concentrations than those determined for the oil. All tested

products are potential fumigant insecticides and can be used to manage *S. zeamais* under storage conditions. Essential oils, especially *G. procumbens* and α-pinene, kill insects for a long time. This is important for controlling *S. zeamais* in stored corn because it helps figure out the shortest time between applications that makes it safe to use as a natural insecticide. The oil of *J. communis* and the compound α-pinene, when used in lethal concentrations for the management of *S. zeamais*, did not influence the germination and vigor of corn seeds.

5. Conclusions

The results obtained in this study demonstrate, for the first time, a comprehensive approach to the use of α-pinene and essential oils in the management of the main stored grain insect pest. The research proved that α-pinene and essential oils from *G. procumbens*, *J. communis*, *P. heptaphyllum*, and *P. pallidum* can control *S. zeamais*. Efficiency data, coupled with product persistence data, will be crucial in the development of more sustainable management methods compared to chemical insecticides. In addition to the effectiveness of the products, it was possible to demonstrate that corn grains remain agriculturally viable, as germination was preserved. Ultimately, the use of essential oils and natural compounds, such as α-pinene, can align with more sustainable approaches in agriculture, potentially minimizing environmental impacts associated with the use of chemical pesticides in the storage environment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14102282/s1, Figure S1A: GCMS chromatogram of *Gaultheria procumbens* essential oil; Figure S1B: GCMS chromatogram of *Juniperus communis* essential oil; Figure S1C: GCMS chromatogram of *Protium heptaphyllum* essential oil; Figure S1D: GCMS chromatogram of *Protium pallidum* essential oil.

Author Contributions: Conceptualization, R.d.C.B.; Methodology, R.d.C.B., L.R.d.S. and D.R.e.S.B.; Software, D.R.e.S.B.; Formal analysis, R.d.C.B., K.d.A.D., D.M.d.A.F.N. and M.O.B.; Investigation, R.d.C.B. and L.F.d.C.; Resources, D.R.e.S.B.; Data curation, R.d.C.B. and L.R.d.S.; Writing—original draft, R.d.C.B. and S.M.d.F.; Writing—review and editing, M.E.O.B., P.R.R.S., L.R.d.S., D.R.e.S.B., M.O.A.I.R., G.N.S., T.B.M.S. and S.M.d.F.; Visualization, M.E.O.B. and G.L.d.S.; Supervision, D.R.e.S.B., M.O.B. and E.K.C.e.S.; Project administration, L.E.d.M.P., G.N.S. and S.M.d.F. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: To the Organic Chemistry Laboratory located at the Natural Science Center—CCN for providing the physical structure.

Conflicts of Interest: The authors declare no conflicts of interest.

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